PAT June, 2018; 14 (1): 125-131 ISSN: 0794-5213
Online copy available at www.patnsukjournal.net/currentissue
Publication of Nasarawa State University, Keffi

Preliminary Assessment of Growth and Seed Production in Advanced Cowpea (*Vigna unguiculata* l. walp.) Lines Infected With Cucumber Mosaic Virus

***Babalola, P. O., Salaudeen, M. T., Gana, A. S. and Muhammad, A. N.**

Department of Crop Production, Federal University of Technology, Minna, Niger State, Nigeria.
**Corresponding author: paulbabalola01@gmail.com*

Abstract

Cowpea (Vigna unguiculata L. Walp) plays a prominent role in the diet of millions of people in developing countries. It is also fed to livestock and contributes substantially towards increased soil fertility. In spite of its numerous economic importance yield is hindered by several pests. Cucumber mosaic virus (CMV) is one of the viruses of economic importance on cowpea. Therefore, 23 advanced cowpea lines were screened for resistance to CMV. Two trials were conducted in a glasshouse using completely randomised design with three replications. Cowpea plants were infected with CMV by sap inoculation at 10 days after sowing. Data were collected on plant growth and seed production. The data were subjected to analysis of variance (ANOVA) and significance of the treatment means was determined at $p \leq 0.05$. Cluster analysis was performed to determine the relationships in growth and seed yields based on Unweighted Pair-Group Method with Arithmetic averages (UPGMA). The cowpea lines 07K-230-2-5, 07K-291-69, 12K-261 and 12K-515 exhibited significant ($p < 0.05$) earliness to flowering with outstanding pod and seed production. These cowpeas flowered at 41-49 days after sowing (DAS), produced a maximum of three pods per plant and between seven and 10 seeds per pod. Cluster analysis indicated that 07K-230-2-5, 07K-291-69, 12K-261 and 12K-515 belonged to the same cluster, indicating genetic similarity. The four (07K-230-2-5, 07K-291-69, 12K-261 and 12K-515) cowpea lines probably contained CMV resistant genes which could be explored for breeding purposes.

***Keywords**: Breeding, Cowpea, Cucumber mosaic virus, Plant growth, Pod and seed production*

Introduction

Cowpea (*Vigna unguiculata* L. Walp), a dicotyledonous plant belongs to the family Fabaceae. It is a major leguminous crop providing food for millions of people in developing countries around the world (Timko and Singh, 2008). In addition, cowpea seeds can be incorporated in various feed formulations and its foliage is a good source of hay for livestock feeding. Cowpea grain contains 28.4 % protein, 1.9 % fat, 6.3 % fibre, 0.00074 % thiamine, 0.00042 % riboflavin and 0.00281 % niacin. It is cultivated extensively in the lowlands and mid-altitude regions of Africa, particularly in the dry savanna. Cowpea is sometimes grown as sole crop but more commonly intercropped with cereal crops such as sorghum and millet (Agbogidi, 2010). According to FAO (2016), cowpea was grown on about 12.3 million hectares of land worldwide, with approximately seven million tonnes of grain yield in 2016. Africa with estimated output of 6.7 million tonnes was the largest producer, obtained from about 12 million hectares.

Up till date, smallholder farmers are the major producers of cowpea in sub-Saharan Africa. Large areas of land are annually cultivated with cowpea but yields are generally low. Low productivity is partly due to infections by viruses. Economically important virus diseases of cowpea are induced by *Blackeye cowpea mosaic virus*, *Cowpea aphid-borne mosaic virus*, *Cowpea yellow mosaic*, *Southern bean mosaic virus*, *Cowpea mottle virus*, *Cowpea golden mosaic virus*, *Cucumber mosaic virus*, *Cowpea mosaic virus* and *Cowpea mild mottle virus* (Alegbejo, 2015). *Cucumber mosaic virus* (CMV) belongs to the genus *Cucumovirus*. It is a single stranded RNA virus with about 29 nm diameter. The thermal inactivation point of CMV varies between 55 and 70 °C. Its longevity *in vitro* ranges from 1-20 days with dilution-end-point of 10^{-3}-10^{-6} (Alegbejo, 2015). Symptoms induced in susceptible cowpea plants include mild to severe leaf mottling, yellowing, mosaic, distortion and stunting. Symptom expression is more intense in mixed infections with *Blackeye cowpea mosaic virus* (BlCMV). The severity of CMV infection depends on the virus strain and resistance background of the invaded cultivar. *Cucumber mosaic virus* can be transmitted mechanically by sap inoculation and through aphids such as *Aphis craccivora*, *A. gossypii* and *Myzus persicae*.

From time immemorial, the best management option against virus diseases is the use of resistant cultivars (Mundt, 2014). It is a strategy that utilizes inherent plants' resistant genes to prevent or restricts the deleterious impacts of infection. Certainly, investigation on the genetic variability and diversity in accessions of cultivated crops could provide vital information for the establishment of breeding programme. Efforts are vigorously geared towards the development of early maturing and high-yielding cultivars in research Institutes such as Institute for Agricultural Research (IAR), Zaria, Nigeria and International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. However, there is need to ensure that recommended cultivars are genetically resistant to virus diseases. Therefore, the objective of this study was to determine the responses of some advanced cowpea lines to CMV.

Materials and Methods

Study location and source of cowpea seeds

The study was conducted in the screenhouse at the Teaching and Research Farm ($9^0$51'N, $6^0$44'E and 212 m above sea level), Department of Crop Production, Federal University of Technology, Minna, Niger State, Nigeria. Minna is located in the Southern Guinea Savanna, agro-ecology of Nigeria. Twenty-three advanced cowpea lines were obtained from International Institute of Tropical Agriculture (IITA), Kano Station, Kano State, Nigeria for the study. These were Ife Brown (susceptible control), TVU 408, 11D-15-40, 04K-267-8, 07K-230-2-5, 07K-291-69, 08K-125-24, 08K-125-107, 08K-193-15, 09K-480, 10K-819-4, 10K-836-3, 12K-261, 12K-515, 12K-809, IT08K-125-100, IT08K-187-5, IT10K-292-10, IT10K-822-7, IT10K-828-3, IT10K-830-9, IT10K-837-1, IT10K-837-1 and IT12K-13. They were selected from the cowpea lines designated for genetic improvement for high yield and pest resistance.

Experimental layout and inoculum multiplication

The twenty-three advanced cowpea lines (treatments) were arranged in Completely Randomised Design (CRD) with three replications. An isolate of CMV previously maintained on silica gels was obtained from the Department of Crop Production, Federal University of Technology, Minna Niger State and propagated in the plants of CMV-susceptible cowpea variety, Ife Brown. Cowpea seeds were sown in five plastic pots (30 cm diameter and 30 cm deep) filled with heat sterilized soil. *Cucumber mosaic virus* inoculum was extracted by grinding the CMV isolate at the rate of 1g/mL in extraction buffer (0.1M sodium phosphate dibasic, 0.1M potassium phosphate monobasic, 0.0l M ethylene diamine tetra acetic acid and 0.00l M-cystine per litre of distilled water) using sterilized mortar and pestle. Two microlitres of β- mercapto-ethanol was added to the extract just before used. Cowpea seedlings were infected with CMV inoculum at 10 days after sowing (DAS) by rubbing the virus extract on the upper leaf surface after dusting with carborundum powder (600- mesh). The inoculated plants were rinsed with distilled water and maintained in a glasshouse. Symptomatic leaves were collected from the inoculated cowpea plants at 3 weeks after inoculation (WAI) and preserved on silica gels at room temperature. These were used for inoculation during the main experiment.

Seedling inoculation, data collection and analysis

Five seeds of each cowpea line were sown in plastic pots (30 cm diameter and 30 cm deep) in a glasshouse and the seedlings were thinned to three plants per pot at seven days after sowing (DAS). Seedlings of the evaluated cowpea lines were inoculated with CMV extract at 10 days after sowing. The procedures for extracting virus and method of inoculation were as described above. Plant height at 3 weeks after inoculation (WAI), number of days to flowering, number of pods per plant and number of seeds per pod were recorded. The data were subjected to analysis of variance (ANOVA) using the general linear model (PROC GLM) procedure of SAS (2008). Duncan Multiple Range Test (DMRT) was used for separating significant ($p \leq 0.05$) treatment mean differences. Cluster analysis was performed to determine the relationships in growth and seed production among the evaluated cowpea lines based on Unweighted Pair-Group Method with Arithmetic averages (UPGMA) (Taamalli *et al.*, 2006).

Results

Effects of *Cucumber mosaic virus* disease on plant height and flowering

Disease symptoms were first observed at 10 days after inoculation (DAI). All inoculated plants in Trials 1 and 2 showed typical foliar symptoms of CMV infection. The symptoms were mild leaf chlorosis and mosaic on the secondary leaves of the infected plants. Generally, substantial height differences were observed among the cowpea lines. Severely infected plants exhibited poor growth and low vigour with characteristic short internodes. In Trial 1, plant heights varied significantly ($p<0.05$) between 34.4 cm and 51.2 cm (Table 1). The plants of IT08K-187-5 were the tallest but the value observed was statistically comparable to that of IT10K-828-3 (49.7 cm). The plants of TVU 408 were the shortest although their heights were not significantly ($p>0.05$) different from those of 12K-515 (35.4 cm). Similarly, the height difference between 09K-480 (46.1 cm) and IT10K-292-10 (47.4 cm) was insignificant ($p>0.05$). Furthermore, 07K-230-2-5 (44.7 cm), 07K-291-69 (44.1 cm), 08K-125-24 (44 cm), 08K-125-107 (43.8 cm), 08K-193-15 (43.9 cm), 10K-819-4 (42.1 cm) and 12K-809 (41.7 cm) also exhibited statistically similar plant heights. The heights of the remaining cowpea lines varied from 35.7 and 39.5 cm but the differences were not significant ($p>0.05$). In Trial 2, the cowpea line 07K-230-2-5 produced the tallest plants of 58.1 cm, followed by TVU 408 (55.1 cm) and IT10K-828-3 (55.2 cm). The cowpea line IT10K-830-9 produced the shortest plants (37.8 cm) while the heights of the remaining lines varied between 38.7 and 53.8 cm.

The evaluated plants exhibited significant ($p<0.05$) differences for number of days to flowering (Table 1). In Trial 1, flowering was earliest in the cowpea line IT08K-187-5 (40 DAS), followed by 12K-261 and 12K-515 which flowered at 41 and 42 DAS, respectively. Flowering was most prolonged in 11D-15-40 which occurred at 57 DAS; which was not significantly ($p>0.05$) different from the observations made on TVU 408 (56 DAS), 04K-267-8 (54 DAS) and IT10K-837-1 (54 DAS). In the remaining cowpea lines, days to flowering ranged between 44 and 52 DAS. Similar trend was observed in Trial 2 where number of days to flowering was earliest in IT08K-187-5 (40 DAS). Other cowpea lines: 12K-261 (42 DAS), 12K-515 (43 DAS), 12K-809 (44 DAS), IT08K-125-100 (45 DAS), IT10K-828-3 (45 DAS), IT10K-292-10 (46 DAS), IT12K-13 (46 DAS) and IT10K-830-9 (47 DAS) also exhibited early number of days to flowering. Conversely, flowering was most delayed in the cowpea line 11D-15-40 (57 DAS) but its time of flowering was statistically similar to that of TVU 408 (56 DAS). Flowering of the remaining cowpea lines was observed between 42 and 55 DAS.

Effects of *Cucumber mosaic virus* disease on number of pods per plant and seeds per pod

Number of pods per plant also differed significantly ($p<0.05$) among the cowpea lines in both trials (Table 1). In Trial 1, 07K-230-2-5, 07K-291-69 and 12K-261 produced the highest number of pods per plant (three pods), followed by 08K-125-24, 08K-125-107, 08K-193-15, 10K-819-4, 10K-836-3, 12K-515, 12K-809, IT08K-125-100, IT08K-187-5, IT10K-292-10, IT10K-828-3 and IT12K-13 which produced two pods per plant each. However, one pod per plant was observed in the remaining cowpea lines. In Trial 2, pod number was significantly ($p<0.05$) highest (three pods per plant) in 07K-230-2-5, 07K-291-69, 08K-193-15 and 12K-515. The cowpea lines 11D-15-40, 08K-125-24, 08K-125-107, 09K-480, 10K-836-3, 12K-261,

2K-809, IT08K-125-100, IT10K-292-10, IT10K-822-7, IT10K-828-3 and IT12K-13 produced two pods per plant, whereas the remaining cowpea lines produced one pod per plant.

The seeds obtained from the severely infected plants were malformed, shrivelled and small irrespective of the cowpea line. In Trial 1, number of seeds varied significantly ($p<0.05$) between four and 10 per pod (Table 1). The cowpea lines 07K-291-69 and 08K-125-107 produced the highest number of seeds per pod (10 seeds). These were followed IT10K-837-1 which had nine seeds per pod. An average of eight seeds was observed in 11D-15-40, 12K-261, 12K-515, 12K-809 and IT10K-828-3 per pod. Similarly, there were seven seeds per plant in 04K-267-8, 07K-230-2-5, 08K-125-24, IT08K-125-100, IT08K-187-5 and IT10K-292-10 while the remaining cowpea lines produced between four and six seeds per pod. As observed in Trial 1, the differences in number of seeds among the cowpea lines were also significant ($p<0.05$). The cowpea line 08K-125-107 exhibited the highest (nine seeds) number of seeds per pod while the lowest (four seeds) was found in Ife Brown and IT10K-822-7. The remaining cowpea lines produced between five and eight seeds per pod.

Cluster analysis revealed that a total of 20 cowpea lines (04K-267-8, 07K-230-2-5, 07K-291-69, 08K-125-107, 08K-125-24, 08K-193-15, 09K-480, 10K-819-4, 10K-836-3, 12K-261, 12K-515, 12K-809, IT08K-125-100, IT10K-292-10, IT10K-822-7, IT10K-828-3, IT10K-830-9, IT10K-837-1, IT12K-13 and Ife Brown) belonged to the same group (cluster 1) (Fig. 1). Within cluster 1, 08K-125-24 and 08K-125-107 were the most genetically identical, whereas 07K-291-69 was closely related to them. The cowpea IT08K-125-100 was the most genetically related to the susceptible check (Ife Brown). Though belonged to the same cluster, Ife Brown was distantly related to 08K-125-24, 08K-125-107 and 07K-291-69. Cluster analysis indicated that 12K-261 and 12K-515 were about 61% genetically related. Members of cluster 1 exhibited plant heights varying between 35.4 and 58.1 cm, flowered between 41 and 55 DAS, majority of them produced three pods per plant, and contained between up to 10 seeds per pod (Fig. 1). On the other hand, 11D-15-40 and TVU 408 constituted cluster 2. They were 34.4 – 55.1 cm tall, flowered at 56 - 57 DAS, produced maximum of two pods per plant, and had up to of eight seeds per pod. The cowpea line IT08K-187-5 was the only member of cluster 3. It exhibited about 47-51.2 cm height, flowered at 40 DAS, had maximum of two pods plant, and produced up to seven seeds per pod (Fig. 1).

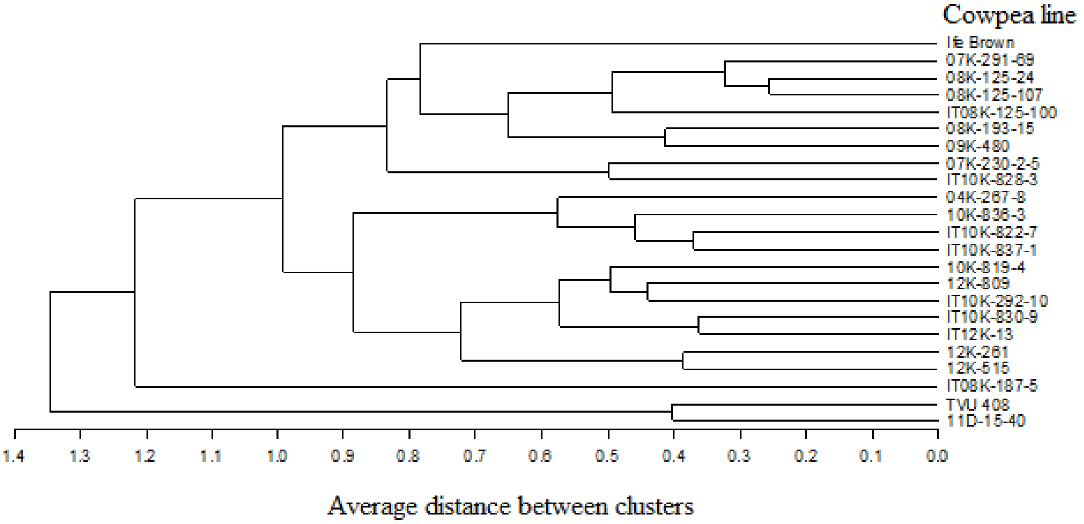

Fig. 1: Dendogram of the relationships in growth and seed production from advanced cowpea lines infected with *Cucumber mosaic virus* inferred from Unweighted Pair Group Method with Arithmetic averages (UPGMA)

Table 1: Growth and seed production of advanced cowpea lines infected with *Cucumber mosaic virus*

	Plant height (cm)		Days to flowering (no.)		Pods per plant (no.)		Seeds per pod (no.)	
Cowpea line	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Ife Brown	35.7^{cd}	50.8^{a-d}	47^{c-f}	47^{c-f}	1^{c}	1^{b}	4^{a}	4^{a}
TVU 408	34.4^{d}	55.1^{ab}	56^{ab}	56^{a}	1^{c}	1^{b}	6^{a}	8^{a}
11D-15-40	38.8^{bcd}	53.8^{abc}	57^{a}	57^{a}	1^{c}	2^{ab}	8^{a}	6^{a}
04K-267-8	37.6^{bcd}	46.9^{a-d}	54^{abc}	55^{ab}	1^{c}	1^{b}	7^{a}	5^{a}
07K-230-2-5	44.7^{a-d}	58.1^{a}	47^{c-f}	47^{c-f}	3^{a}	3^{a}	7^{a}	7^{a}
07K-291-69	44.1^{a-d}	49.5^{a-d}	49^{b-e}	49^{b-e}	3^{a}	3^{a}	10^{a}	8^{a}
08K-125-24	44.0^{a-d}	48.6^{a-d}	46^{def}	47^{c-f}	2^{b}	2^{ab}	7^{a}	8^{a}
08K-125-107	43.8^{a-d}	47.2^{a-d}	47^{c-f}	47^{c-f}	2^{b}	2^{ab}	10^{a}	9^{a}
08K-193-15	43.9^{a-d}	47.5^{a-d}	51^{a-d}	50^{a-d}	2^{b}	3^{a}	6^{a}	7^{a}
09K-480	46.1^{ab}	51.6^{a-d}	52^{a-d}	52^{abc}	1^{c}	2^{ab}	4^{a}	6^{a}
10K-819-4	42.1^{a-d}	44.2^{a-d}	48^{cde}	48^{c-f}	2^{b}	1^{b}	4^{a}	5^{a}
10K-836-3	37.6^{bcd}	44.5^{a-d}	51^{a-d}	49^{b-e}	2^{b}	2^{ab}	6^{a}	8^{a}
12K-261	37.9^{bcd}	38.7^{cd}	41^{ef}	42^{fg}	3^{a}	2^{ab}	8^{a}	7^{a}
12K-515	35.4^{d}	43.1^{bcd}	42^{ef}	43^{efg}	2^{b}	3^{a}	8^{a}	7^{a}
12K-809	41.7^{a-d}	41.4^{bcd}	46^{def}	44^{d-g}	2^{b}	2^{ab}	8^{a}	6^{a}
IT08K-125-100	39.5^{bcd}	48.0^{a-d}	44^{def}	45^{d-g}	2^{b}	2^{ab}	7^{a}	8^{a}
IT08K-187-5	51.2^{a}	47.0^{a-d}	40^{f}	40^{g}	2^{b}	1^{b}	7^{a}	5^{a}
IT10K-292-10	47.4^{ab}	41.4^{bcd}	47^{c-f}	46^{c-g}	2^{b}	2^{ab}	7^{a}	6^{a}
IT10K-822-7	38.2^{bcd}	40.4^{bcd}	51^{a-d}	50^{bcd}	1^{c}	2^{ab}	6^{a}	4^{a}
IT10K-828-3	49.7^{a}	55.2^{ab}	44^{def}	45^{c-g}	2^{b}	2^{ab}	8^{a}	7^{a}
IT10K-830-9	38.6^{bcd}	37.8^{d}	48^{c-f}	47^{c-f}	1^{c}	1^{b}	4^{a}	8^{a}
IT10K-837-1	38.8^{bcd}	40.7^{bcd}	54^{abc}	52^{abc}	1^{c}	1^{b}	9^{a}	6^{a}
IT12K-13	38.2^{bcd}	40.8^{bcd}	45^{def}	46^{c-g}	2^{b}	2^{ab}	5^{a}	6^{a}
± SEM	2.0	2.9	1.6	1.4	0.1	0.1	1.3	1.1

Means followed by different letter (s) in the same column are significantly different at 5 % probability level of significance according to Duncan Multiple Range Test (DMRT)

Discussion

Hunger and malnutrition afflict more than half of the developing world's population, especially women and preschool children. In 2016, the population of undernourished people was estimated at 815 million, which was relatively higher than 777 million reported in 2015 (FAO, IFAD, UNICEF, WFP and WHO, 2017), and majority of them live in developing countries. Furthermore, 155 million children under five years of age across the world suffer from stunted growth. It is also on record that nearly two-third of all deaths of children are induced by nutritional deficiencies. Apart from this, about two billion people across the world suffer from another type of hunger known as "hidden hunger," which is caused by an inadequate intake of essential micronutrients in the daily diet despite increased food crop production. Cowpea is capable of supplying some of the essential micronutrients, minerals and protein required for human normal growth and development (Okonya and Maass, 2014).

The responses of the evaluated cowpea lines revealed their genetic variability. Heterogeneous plant materials are sources of growth and yield traits that can be explored for breeding desired crop varieties. Although all the cowpea lines flowered in less than two months after sowing, indicating earliness to maturity, identification of extra early maturing cowpea lines would be more desirable especially in sub-Saharan Africa where climate change is already worsening food crisis (Salaudeen *et al*., 2016). The symptoms observed on the infected plants are consistent with those documented by Alegbejo (2015). Leaf chlorosis and mosaic were the consequences of CMV infection. Viruses are obligate parasites that normally depend on attacked plants for replication and establishment. The development of CMV disease symptoms corroborated the findings of Arogundade *et al*. (2010) who observed differential responses in soyabean lines to CMV. Number of pods per plant is an important quantitative trait in cowpea because of its direct relationship with seed production. The cowpea lines which produced one pod per plant were worse affected by the virus. The production of two or three pods in some cowpea lines implied some level of tolerance to CMV. Tolerance is a manifestation of mild level of infection. Owing to scarcity of immune varieties cultivation of tolerant varieties remain an alternative strategy against most virus diseases. Studies have shown that viruses cause epidemics on all major crops and pose serious threat to global food security. Being strict intracellular pathogens, viruses cannot be controlled chemically and prophylactic measures rely on the destruction of infected plants and excessive pesticide applications to limit the population of vector organisms. However, use of crop genetic resistances which relies on mechanisms governing plant–virus interactions is widely employed (Nicaise, 2014).

Seed production differed significantly as a result of substantial variation in pod numbers among the evaluated cowpea lines. Number of seeds per pod is one of the important attributes for consideration during cowpea breeding. Thus, the cowpea lines such as 07K-230-2-5, 07K-291-69, 12K-261 and 12K-515 which produced appreciable pod and number of seeds per pod under CMV disease probably possessed resistant genes. Poor pod and seed production in some cowpea lines could be attributed to deleterious impact of the virus. This corroborates the findings of Kareem and Taiwo (2007) who reported severe retardation in pod and seed numbers for cowpea cultivars infected with Cowpea aphid-borne mosaic virus, Cowpea mottle virus and Southern bean mosaic virus at 10 DAS. Some cowpea lines produced maximum of eight seeds per pod but they were characterized by low pod yield, indicating negative correlation. Pod and seed yields are quantitative character under the influence of several genes. The genes controlling such traits may be located on different chromosome and not operate synergistically.

Conclusion and Recommendations

This study revealed the tolerance some evaluated cowpea lines to CMV infection. Although they were all early maturing the cowpea lines 07K-230-2-5, 07K-291-69, 12K-261 and 12K-515 exhibited significant ($p<0.05$) earliness to flowering with outstanding pod and seed production. The four (07K-230-2-5, 07K-291-69, 12K-261 and 12K-515) cowpea lines probably contained CMV resistant genes which could be explored for breeding purposes.

References

Agbogidi, O. M. (2010). Response of six cultivars of cowpea (*Vigna unguiculata* (L.) Walp.) to spent engine oil. Afr. J. Food Sci. Tech., 1:139-142.

Alegbejo, M. D. (2015). Virus and virus-like diseases of crops in Nigeria. Ahmadu Bello University Press, Zaria, Nigeria. Pp. 272.

Arogundade, O., Balogun, O. S., Shokalu, O. and Aliyu, T. H. (2010). Influence of *Cowpea mottle virus* and *Cucumber mosaic virus* on the growth and yield of six lines of soybean (*Glycine max* L.Merrril). J. Agric. Sci., 2: 72–78.

FAO (Food and Agriculture Organization) (2016). Cowpea production. Available from: http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

FAO, IFAD, UNICEF, WFP and WHO (2017). The State of Food Security and Nutrition in the World. Building resilience for peace and food security. Rome.

Kareem, K. T. and Taiwo, M. A. (2007). Interactions of viruses in cowpea: effects on growth and yield parameters. Virol. J., 4:15.

Mundt, C. C. (2014). Durable resistance: A key to sustainable management of pathogens and pests. Infect. Genet. Evol., 27: 446–455.

Nicaise, V. (2014). Crop immunity against viruses: Outcomes and future challenges. Front. Plant Sci., 5: 1 – 18.

Okonya, J. S. and Maass, B. L. (2014). Protein and iron composition of cowpea leaves: An evaluation of six cowpea varieties grown in Eastern Africa. Afr. J. Food, Agric. Nutrition Dev., 14:2129 – 2140.

SAS (Statistical Analysis System). (2008). Statistical analysis system SAS/STAT User's guide. ver. 9.2. Cary: N.C SAS Institute Inc.

Salaudeen, M. T., Adama, C. J., Abdullahi, A. A., Ayeleke, D. A. and Ibrahim, A. D. (2016): Climate change and viral diseases in relation to crop productivity and food security: A Review. Int. J. Appl. Biol. Res., 7: 56 – 65.

Taamalli, W., Geuna, F., Banfi, R., Bassi, D., Daoud, D. and Zarrouk, M. (2006). Agronomic and molecular analyses for the characterisation of accessions in Tunisian olive germplasm collections. Electron. J. Biotech.,. 9:467–481.

Timko, M. P. and Singh, B. B. (2008). Cowpea, a multifunctional legume. In: *Genomics of Tropical Crop Plants* (Moore, P.H. and Ming, R. eds.). Springer Science, New York, pp. 227 – 258.